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Review paper

APPLICATION OF SELDI-TOF-MS IN PROTEIN PROFILING: PROMISES AND PITFALLS

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Summary: Serum protein profiling by Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF-MS) appears to be an important diagnostic tool for a whole range of diseases. Sensitivities and specificities obtained with this new technology often seem superior to those obtained with current biomarkers. However, reproducibility and standardization are still problematic. The present review explains the SELDI-TOF-MS technique and discusses some important aspects for proteomics studies, like pre- and post-analytical aspects and quality control procedures. Own data about biomarker discovery for the diagnosis of sarcoidosis are also presented in this review.

Key words: pre-analytical aspects, post-analytical aspects, proteomics, reproducibility, sarcoidosis, SELDI-TOF-MS, quality control

Introduction

The field of proteomics has developed rapidly in recent years. Until the mid 1990s scientists studied individual genes and proteins or a handful of biologically related genes and proteins. The point of proteomics is to characterize the behaviour of the system, rather than the behaviour of any single component. The proteome is dynamic and in constant flux due to a combination of factors. These factors include posttranslational modifications and functional regulation of gene expression (1). In proteomics, analyses are directed at complex mixtures of proteins and identification is not necessarily performed by complete sequence analysis but can also be performed by partial sequence analysis with the aid of database matching tools.

There are different techniques for the expression analysis of proteins, like two-dimensional electrophoresis (2-DE) combined with Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS), and/or Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS). The present review is focused on the SELDI-TOF-MS technique.

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SELDI-TOF-MS

There have been many reports on the application of SELDI-TOF-MS technology since its first introduction in 1993 by Hutchens and Yip (2). SELDI-TOF-MS is an approach that tries to overcome the requirements for purification and separation of proteins prior to mass spectrometry analysis (3). It is a novel approach to biomarker discovery that combines two powerful techniques: chromatography and mass spectrometry. One of the key features of SELDI-TOF-MS is its ability to provide a rapid protein expression profile from a variety of biological and clinical samples (4). It consists of selective protein extraction and retention on chromatographic chip surfaces and their subsequent analysis by a simple laser desorption ionization mass spectrometer (5). It differs in several aspects from conventional MALDI-TOF-MS. For MALDI-TOF-MS, analytes are directly spotted onto a plate. This is usually a metal plate. The applied samples are usually tryptic digests from proteins separated by 2-DE, although proteins purified by other separation methods are also compatible with the method. Before the deposition of

analytes, the energy absorbing matrix (EAM) is placed on the plate or mixed in with the sample. The matrix will absorb energy from the laser causing the analytes to be ioni-zed by MALDI-TOF-MS (6)

ProteinChip arrays

For the SELDI-TOF-MS technique different ProteinChip arrays (Ciphergen Biosystems Inc.) are used. The chromatographic surfaces that make up the various ProteinChip arrays are uniquely designed to retain proteins from a complex sample mixture according to specific properties such as hydrophobicity, charge, etc (4) (*Figure 1*). The procedure for detecting protein biomarkers is very simple. A few microliters of the sample are dispensed onto the ProteinChip surface under specific binding conditions that determine which proteins will be retained by the surface. Protein specificity is achieved through the application of a series of washes with an appropriate solvent or buffer designed to elute unbound proteins and interfering substances, such as salts, detergents, lipids. Only proteins actively interacting with the spot surfaces are analyzed in the Protein Biosystem series instrument (Ciphergen Biosystems Inc.) because all other components are washed off in advance. One of the most obvious advantages of this surface enhanced process is that components such as salts, detergent, or lipids which commonly cause problems with other analytical tools are washed away as part of the SELDI process (7).

By choosing different ProteinChip arrays with array-specific surface components, different proteins will be analyzed depending on the chip characteristics. In fact, the interaction of the analyte and the chip introduces a purification step. Each combination of ProteinChip array types together with the binding and washing buffers of choice results in a unique binding capacity for a special subset of peptides and proteins. After the addition of sample and washing buffers, the EAM is applied to the ProteinChip array. The EAM will facilitate desorption and ionization in the PBS series instruments.

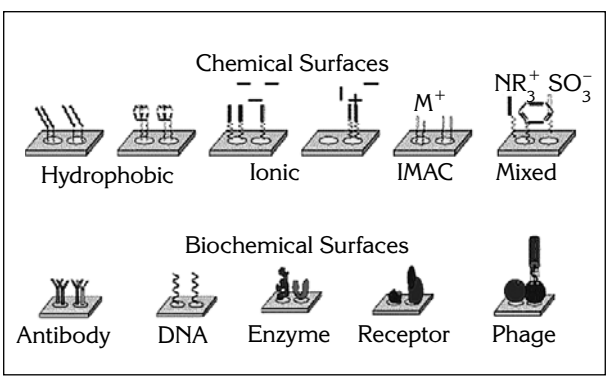

Figure 1 The different types of ProteinChip arrays. The chemical surfaces are chromatographic ProteinChip arrays with hydrophobic, cationic, anionic, metal ions for immobilized metal affinity binding (IMAC) or hydrophilic spots. The biochemical surfaces are designed for the coupling of biomolecules in antibody-antigen assays, DNA-protein binding experiment, coupling of enzymes, receptor-ligand interaction and for coupling of phages.

Desorption/Ionization process

After introducing the ProteinChip array into the ProteinChip Reader, a laser beam is directed onto the sample on the spot. Upon laser activation, the sample becomes irradiated and the desorption and ionization proceed to liberate gaseous ions from the ProteinChip arrays. These gaseous ions enter the TOF-MS region of the instrument, which measures the mass-to-charge ratio (*m/z*) of molecular ions of each protein based on its velocity through an ion chamber (4). The time-of-flight corresponds inversely to the *m/z* value. As a first result, the molecules in the sample are represented in a graph with the *m/z* value on the x-axis and the corresponding signal intensity on the y-axis (7) (*Figure 2*).

Advantages of SELDI-TOF-MS

SELDI-TOF-MS has numerous advantages over other methods such as 2-DE combined with MALDI-TOF-MS and/or LC/MS/MS. SELDI-TOF-MS has a much higher throughput capability, requires significantly lower amounts of the sample, offers higher resolution at low mass ranges, and is easy to use (8). SELDI-TOF-MS can effectively resolve polypeptides and peptides smaller than 20 kDa (9). The 2-DE approach, where proteins are first sepa-rated by their isoelectric point and subsequently by their molecular weight, was developed to increase the resolving power for the analysis of complex protein mixtures. Whereas the enhanced resolution of 2-DE gels contributed greatly to our understanding of the wide variety of proteins in a given sample, it still includes the disadvantage of the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) method of giving preference to the most abundant proteins. In addition,

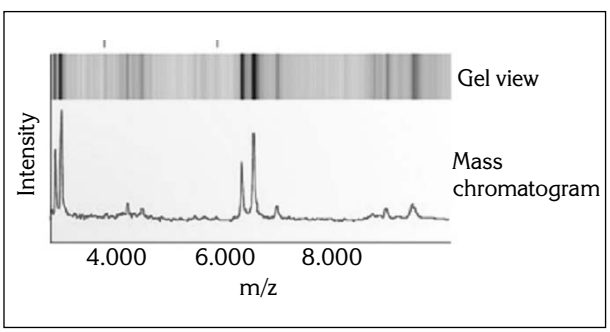

Figure 2 The ions of the molecules in the sample are represented in a graph (see mass spectrum) with the mass-to-charge ratio (*m/z*) on the x-axis and the corresponding signal intensity on the y-axis. It can also be presented in a gel view (see *gel view*).

proteins in the peptide range as well as those of high hydrophobicity or of extreme isoelectric points are typically neglected, resulting again in a loss of potentially interesting proteins (10). 2-DE is labour intensive, time consuming, and difficult to standardize among laboratories (4).

The high throughput ability of the SELDI-TOF-MS system allows hundreds of samples to be screened for disease biomarker identification in a relatively short time period, providing investigators the opportunity to compare patient-to-patient variability (4). SELDI-TOF-MS is a recently established improvement on some of the concepts of MALDI-TOF-MS. ProteinChip arrays allow researchers to purify and detect a subset of proteins in the sample at the same time by using a variety of surface chemistries such as classic chromatographic surfaces (e.g., cation/anion exchanges) and biologically activated surfaces to capture specific molecular counterparts. This benefit is especially effective for biological samples such as body fluids and conditioned medium like tissue homogenate containing a variety of proteins (11).

Biomarker Discovery

The true scientific goal of serum proteomic pattern analysis is, in fact, biomarker discovery. However, since the study by Petricoin et al. (12) on proteomic patterns to detect ovarian cancer, the use of SELDI-TOF-MS protein profiling as a diagnostic tool has become an important subject of investigation, too (13). Until now, this approach has been suggested for different diseases, for example ovarian (12, 14–18), prostate (9, 19–22) and lung (23) cancer, but also for inflammatory diseases (24, 25).

Currently, the pipeline from translation of new biomarkers into tests appears to have a bottleneck at the early stages of translation of research markers into clinical tests. Research groups performing discovery and clinical studies rarely have the resources to develop prototype analyzers or test reagent sets, to manufacture them, or to proceed with other steps in commercialization. These steps usually rely on the *in vitro* diagnostics industry, which has had relatively low investments in the development of new markers. Beside the development of tests, there is the need for evaluation in clinical laboratories, submission for approval by the US Food and Drug Administration (26), establishment of reimbursement rates by the Medicare system and insurers, and education of physicians about tests ordering and interpretation. The process of translating new markers into clinical laboratory test entails contributions from multiple disciplines, including scientists; engineers; business, legal, and regulatory professionals; clinicians; and clinical chemists (27).

Pre-analytical aspects

Protein profiling can only become a reliable diagnostic tool when it fulfills the criteria for reproducibility and standardization that are generally accepted for diagnostic tests in clinical chemistry. Therefore, the present review discusses pre-analytical aspects, supplemented with some own data, which are essential for reproducibility and standardization.

Storage effects

To avoid pre-analytical errors, sample collection for proteomic analysis should be accurately described and standardized. Effects of sample storage and the consequences of differences in sample preparation are highly underestimated. We have recently compared protein profiles of freshly frozen serum samples with frequently thawed serum samples. The samples were thawed at least 8 times and were stored at –80 °C (28). Freshly frozen serum and frequently thawed serum from 8 sarcoidosis patients and 8 healthy controls were spotted on a CM10 (cation exchange) and on a NP20 (normal phase) ProteinChip array. In the frequently freeze-thawed serum three peaks were detected, allowing clear discrimination ($p < 0.05$) of sarcoidosis from healthy controls using the CM10 chip (*m/z* values: 3808 (up-regulation in sarcoidosis), 4277 (down-regulation in sarcoidosis), 8932 (up-regulation in sarcoidosis)). However, exactly the same experiment using freshly frozen serum no longer allowed us to discriminate between sarcoidosis and controls with the 3 *m/z* units, described above, because the peak differences were not significant. In contrast, in the freshly frozen samples only one significant peak at *m/z* 8702 was found. This peak was different from the ones found in the frequently thawed samples. The fact that another single marker was found on the CM10 indicates that it concerns freeze-thaw artefacts in frequently thawed serum samples and underlines the importance of standardization (28). This apparently trivial example clearly shows that, for protein profiling studies, control and patient samples should be treated and stored under exactly the same conditions.

It is to be expected that, especially in the earlier protein profiling studies, archived samples were used for which conditions of control and patient populations were not fully identical. It has now become apparent that both the number of freeze-thaw cycles, freezing temperature and storage time should at least be identical for both study and control population. This can easily be overcome in prospective studies by dividing the samples in aliquots before storage.

Serum or plasma

Until now, insufficient information is available to decide whether serum or plasma should be preferred in proteomic studies. Most studies have used serum, but further research on this topic is required. Therefore, in a recent study we compared serum and EDTA plasma with and without protease inhibitors (28). Serum and plasma samples from 8 sarcoidosis

Table I Serum and EDTA plasma samples with and without protease inhibitors were spotted on CM10 and NP20 ProteinChip arrays. The mean number of peaks in the protein spectra (*m/z* range of 2,500 to 150,000) from 8 sarcoidosis and 8 healthy control samples are indicated.

	CM10 (N peaks)	NP20 (N peaks)
Serum	64	58
Serum with protease inhibitors	63	63
EDTA plasma	28	11
EDTA plasma with protease inhibitors	28	14

patients and 8 healthy controls were spotted on CM10 and NP20 ProteinChip arrays. The mean protein peaks in serum with and without protease inhibitors were compared with the mean protein peaks in plasma with and without protease inhibitors (see *Table I*). *Table I* shows that in the *m/z* range of 2,500 to 150,000 serum without protease inhibitors showed slightly more protein peaks (n=64) than serum with inhibitors (n=63), but EDTA plasma with and without protease inhibitors was clearly inferior (both n=28) on the CM10 ProteinChip array. On the NP20 ProteinChip array serum without protease inhibitors showed slightly fewer protein peaks (n=58) in the same *m/z* range than serum with inhibitors (n=63), but EDTA plasma with (n=11) or without (n=14) protease inhibitors was evidently inferior.

More significant peaks which could discriminate sarcoidosis from healthy control samples were found in the serum samples with and without protease inhibitors compared to plasma samples with and without protease inhibitors on the CM10 and NP20 ProteinChip arrays. It is generally assumed that more peaks can lead to more significant differences between populations, as was the case in our study. Theoretically, however, plasma with protease inhibitors contains more intact proteins not attacked by proteolytic enzymes. Further examinations on the differences between serum and plasma are required.

Sample preparation

Samples can be denatured with urea/CHAPS (9, 14, 16–20, 22), but can also be fractionated with anion exchange chromatography (15). Denaturing conditions allow protein-protein interaction disruption before analysis by SELDI-TOF-MS. With fractionation by anion exchange chromatography the highly abundant proteins such as albumin and immunoglobulins (60–80% of total serum protein content), which can interfere with the resolution and sensitivity of the proteome profiling techniques, will be visible in specific fractions. The albumin signal will be mainly visible in fraction 3 (pH 5), fraction 4 (pH 4) and fraction 5 (pH 3). In the same way, the immunoglobulins signals will be observed in fraction 1 (pH 9) and fraction 2 (pH 7). In this procedure, the highly abundant proteins are not removed, but they are localized to one or a few particular fractions (29). Linke et al. (30) illustrated that fractionation greatly increases the number of peptide and protein ion signals that can be observed by SELDI-TOF-MS, when compared to both unfractionated (only denatured) as well as albumin-depleted samples. By using different denaturing steps or using fractionated samples, other significant peaks resulting in different biomarkers can be detected.

Sampling time

It is known that the serum concentration of certain proteins is influenced by the sampling time, i.e. time between puncture and storage (clotting time, spinning time and time between spinning and storage). However, the type of material also plays a role. For instance, B-type natriuretic peptide (BNP), a well-known marker for heart failure, is unstable in the serum as a result of the presence of proteolytic enzymes. The degradation progresses even during storage at –20 °C and can only be prevented by addition of protease inhibitors or by measuring plasma BNP instead of serum BNP (31). The information on sampling time is often not clearly indicated in different studies. This can be problematic when archived samples are used. However, in prospective proteomic studies sampling time should be standardized.

We suggest, according to the World Health Organization (WHO): Anticoagulants in diagnostic laboratory investigations to use a clotting time of 30 minutes at room temperature, spinning for 15 minutes at a minimum speed of 1500g, and storage of the samples in aliquots within 1 hour at –80 °C after blood collection. Obviously, the consequences of differences in sample characteristics within a study population, but also between study and control population like for instance use of fasting or non-fasting samples, age-matching of the samples, should always be properly standardized.

Patient population

The number of patients and healthy controls in the training and validation sets is very important because the reliability of the results improves with increasing numbers. A clear description of the training and validation population is essential, like that of the severity of disease. Because SELDI-TOF-MS fingerprinting probably measures peptides present in high abundance in serum (e.g. mg/L to g/L range), the molecules which are detected probably originate from common disease mechanisms or general protection mechanisms, i.e. epiphenomena of the diseases, such as acute phase response, cachexia etc. It is clear that the robustness of the technology should be validated by comparing patient groups with comparable disease mechanisms. Method validation should therefore be extended not only to healthy controls, but also to

diseases with comparable generalized disease conditions (infection, cachexia etc).

Post-analytical aspects

Bioinformatics and biostatistics

Peak detection, laser settings and data analysis software affect the ultimate *m/z* values found. Different multivariate analysis software can be used to classify different groups. Biomarkers Patterns (Ciphergen Biosystems Inc.) is a decision tree algorithm which is very often used in protein profiling studies. The decision trees can be based on the intensity, S/N ratio or Area Under Curve (AUC). Propeak, Classification and Regresion Tree (CART), AdaBoost, and principal component analysis (PCA) are other examples of multivariate analysis software programs which can be used to classify different groups. Some groups develop their own statistical software program by combining more multivariate analysis techniques. It is hard to compare the results of studies when all these different kind of software programs are used to classify groups.

In a recent review we showed that, apart from the pre-analytical strategy, the post-analytical strategy also has an enormous impact on the final results. By comparing previous reports on prostate and ovarian cancer, we showed large differences in *m/z* values of the biomarkers presented in different studies, even in studies with comparable patient populations (28). It should be noted that careful and precise selection of the peak labeling settings and normalization of peak intensities are considered critical for biomarker identification and for the efficient and reliable performance of any learning algorithm used in conjunction with the SELDI-TOF-MS system (16).

Potential biomarkers for the diagnosis of sarcoidosis

In our research group we try to discover novel biomarkers for different kinds of diseases, like sarcoidosis, Ankylosing Spondylitis (AS), Multiple Sclerosis (MS), and small vessel disease. In this review the most important findings of a study on sarcoidosis will be discussed.

Sarcoidosis is a multi-systemic inflammatory disorder, which affects the lungs in 90 percent of the cases. The main pathologic feature is chronic inflammation resulting in non-caseating granuloma formation. Until now there is no satisfying biomarker for the diagnosis or prognosis of sarcoidosis. The study is focused on the detection of potential biomarkers in serum for the diagnosis of sarcoidosis using SELDI-TOF-MS.

Table II The *m/z* values of the peak splitters, the cut-off values of the peak intensities, the p-values and the results of the learn and test set for the sarcoidosis and control samples per laser setting. The spectra were calibrated with the protein low calibration on the NP20 array.

Laser setting	*m/z* value protein-low calibration (NP20)	Cut-off peak intensity	p-value	Correct classification of the learn set	Correct classification of the test set
LI 220 SE 7	11,955	1.113	0.003	S : 30/35 = 86% C : 23/35 = 66%	S : 30/35 = 86% C : 22/35 = 63%
LI 190 SE 7	11,734 17,377	3.060 0.450	0.01 0.05	S : 26/35 = 74% C : 29/35 = 83%	S : 26/35 = 74% C : 25/35 = 71%

LI: laser intensity; SE: detector sensitivity; S: sarcoidosis; C: control

Table III ROC curve analysis results for the inflammatory markers, ACE and sIL-2R

	AUC (95% confidence intervals)	Selected cut-off	Sensitivity (%)	Specificity (%)
ACE	0.779 (0.668–0.891)	16.5	70.6	71.4
SIL-2R	0.667 (0.539–0.795)	515.5	62.9	57.1

ACE: Angiotensin Converting Enzyme; AUC: area under curve; ROC: receiver operating characteristic; sIL-2R: soluble Interleukin-2 Receptor

For the detection of potential biomarkers, protein profiles of anion exchange fractionated serum of 35 sarcoidosis patients and 35 healthy controls were compared using SELDI-TOF-MS. Sensitivities and specificities of the potential biomarkers obtained with SELDI-TOF-MS, generated with decision tree algorithm, were compared to the conventional markers Angiotensin Converting Enzyme (ACE) and soluble Interleukin-2 Receptor (sIL-2R).

Optimal classification was achieved with metal affinity binding (IMAC) arrays coupled with copper sulphate. A single marker with an ion at *m/z* 11,955 resulted in a sensitivity and specificity of 86% and 63% respectively. A multimarker approach of two peaks, *m/z* values of 11,734 and 17,377, resulted in a sensitivity and specificity of 74% and 71% respectively (*Table II*). The SELDI mass spectra and gel views of the three potential biomarkers are illustrated in *Figure 3*. These sensitivities and specificities were higher compared to measurements of ACE and sIL-2R (*Table III*). Identification of the peak at *m/z* 17,377 resulted in the alpha chain of haptoglobin. In *Figure 4* is illustrated that the band at a molecular weight of 17 kDa, which represents the peak at *m/z* 17,377, is not visible in the sarcoidosis patient sample (*Figure 4A*), but clearly visible in the healthy control sample (*Figure 4B*).

This study acts as a proof-of-principle for the use of SELDI-TOF-MS in the detection of new biomarkers for sarcoidosis. The peak of the multimarker at *m/z* 17,377 was successfully identified as the alpha chain of haptoglobin, but the identification of potential biomarkers at *m/z* 11,734 and 11,955 appeared difficult. Further attempts will be undertaken because the identification of these two markers will lead to a better understanding of sarcoidosis and would be of great interest.

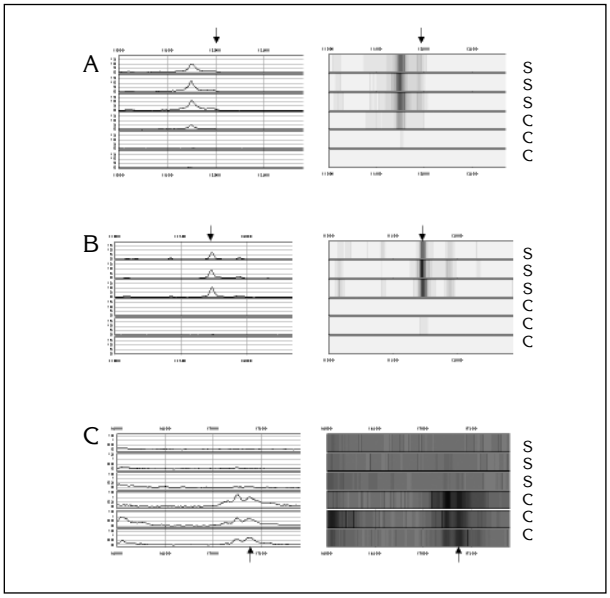

Figure 3 SELDI-TOF-MS spectra and gel views of the three potential biomarkers at *m/z* 11,955 (A), 11,734 (B), and 17,377 (C). Representative protein spectra of three sarcoidosis (S) and three control (C) samples are illustrated per marker. The marker is indicated with a vector. The mass is given as *m/z* value on the x-axis and the intensity is displayed along the y-axis.

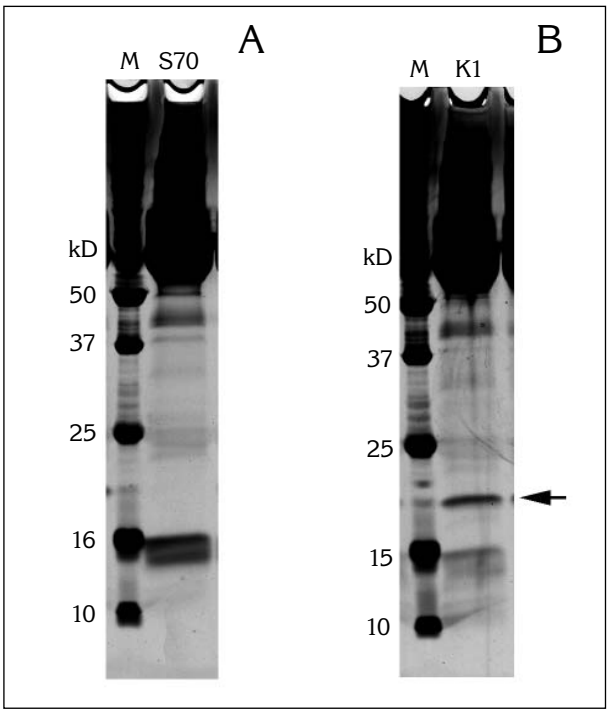

Figure 4 Silver stained band pattern of one fraction 3 sarcoidosis sample (A) and one fraction 3 healthy control sample (B) separated by SDS-PAGE. The left lane shows the marker and the right lane shows the sarcoidosis sample (S70) in Figure 4 A and the healthy control sample (K1) in Figure 4 B. In Figure 4 A there is no band visible at a molecular weight of 17 kDa. Figure 4 B clearly shows a band at 17 kDa and this band is indicated with a vector.

Quality control

As mentioned before, the effect of pre- and post-analytical variables on protein profiling needs further and more systematic investigation. Therefore, a stringent standardized protocol is needed, not only for pre- and post-analytical aspects, but also for calibration and quality control (QC) performance. This review describes the results of the quality control procedure which was described in a previous report of Bons et al. (32).

Recently, Plebani et al. (33) indicated that only few published studies carefully described the quality control procedures incorporated in proteomic experimental protocols. The aim of our study was to establish a well-defined protocol for the calibration of Protein Biosystems IIc (PBS IIc) instrument, to implement QC samples with independent certified standards and to determine acceptance criteria for quality control. Because the QC samples were spotted on a NP20 array, which is a normal phase array, without

washing or selective binding steps, only the MALDI-TOF-MS part of the PBS IIc instrument was checked. Stable instrument performance over time is a prerequisite before any proteomic experiments should be performed. The QC procedure described in our report acts prospectively by checking the calibration every week in contrast to some other studies, where QC samples are included in the profiling studies and quality control thus acts retrospectively or where no quality control procedure is performed at all.

Acceptance criteria

Data analysis was performed with in house developed software (ShewhartPlots), which was based on the Shewhart control chart principle (34). The following parameters were imported in this software: *m/z* values, intensities, signal-to noise (S/N) ratios and peak resolutions. Two-dimensional Youden plots were made by drawing insulin (x-axis) and apomyoglobin (y-axis) in one plot for all parameters and three--dimensional Youden plots were made by drawing insulin (x-axis), apomyoglobin (y-axis) and albumin (z-axis) in one plot for all parameters (*Figure 5*). After measuring the QC samples for a longer period, we concluded that most data points were within the process mean ± 2 standard deviations (SD) and none of the points were outside the process mean ± 3 SD range. On the basis of those results, we defined the following acceptance criteria: data points should be in

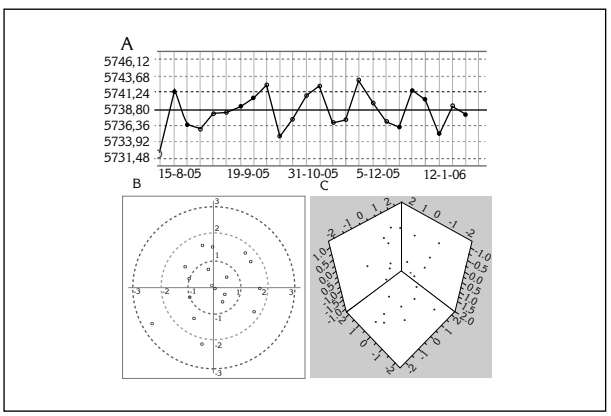

Figure 5 Examples of a graphic and Youden plots for the *m/z* values, S/N ratios and peak resolutions generated with the in house developed ShewhartPlots. The process mean and the standard deviation (SD) values (+ 1, 2 and 3 × SD, -1, 2 and 3 × SD) of the *m/z* values of insulin are indicated (A). The *m/z* values are indicated on the x-axis and the SD values are indicated on the y-axis. The two dimensional Youden plot of the S/N ratios of insulin and apomyoglobin is illustrated (B). The SD values (+ 1, 2 and 3 × SD, -1, 2 and 3 × SD) of the S/N ratios of the insulin and apomyoglobin are indicated on the x- and y-axis, respectively. The three dimensional Youden plot of the peak resolutions of insulin, apomyoglobin, and albumin are shown (C). The SD values of the peak resolutions of insulin, apomyoglobin, and albumin are indicated on the x-, y-, and z-axis, respectively.

the established range of the process mean ± 2 SD for the *m/z* values, peak intensities, S/N ratios, and peak resolutions for insulin, apomyoglobin and albumin in the QC samples. The following Westgard rules need to be fulfilled: 1_{3s}, 2_{2s}, 4_{1s}, 8_{x}, 10_{x}, and 12_{x} (35). Because we work with a process mean, the mean will change when a new data point is added in comparison to a fixed mean, where the mean is always constant. Using a new batch of QC samples can result in a difference between the process mean and the 2 SD range for the *m/z* values, intensities, signal-to-noise (S/N) ratios and peak resolutions of the QC samples.

Reproducibility

We also showed that variations in the signal of the QC samples can be caused by pipetting variability in the handling of the QC sample, spot and chip variability, crystallization of the EAM and laser detector variability over time.

The reproducibility of serum protein profiling by SELDI-TOF-MS was investigated by spotting one QC sample consisting of insulin and apomyoglobin on 2 or 4 NP20 chips. Coefficient of variation (CV) values from approximately 10 to 40% were achieved for intensities and signal-to-noise (S/N) ratios. The pooled CV value for the mass accuracy was below 0.1%. The CV values for intensities, S/N ratios and mass accuracy described in the study of Bons et al. (32) were comparable with the CV values reported by Semmes et al. (36). Semmes et al. performed across-laboratory measurement of three *m/z* peaks in a standard pooled serum. This resulted in a 0.1% CV for mass accuracy. The CVs for signal-to-noise ratios were 34–40% and the variations in the intensities of the three peaks for all laboratories were 15–36%

Lee et al. (37) also indicated that it is hard to reproduce experiments. They investigated renal cell carcinoma and included samples from patients with renal cell carcinoma, patients with benign urological diseases and healthy controls in the training set. An initial blind group of samples was used to test the models. Sensitivities and specificities of 81.3–83.3% were achieved. However, subsequent testing 10 months later with a different blind group of samples resulted in much lower sensitivities and specificities (41.0–76.6%).

Potential sources of variability that arise during SELDI-TOF-MS profiling include spot-to-spot variation of chip surfaces, laser detector variability over time, pipetting variability (38) and the crystallization process of the EAM (39, 40). We demonstrated that the reproducibility of the crystallisation process can be increased by using an incubator with a constant temperature of 28 °C and a constant atmospheric humidity of 45%. The same QC sample (insulin and apomyoglobin) as described above was used and CV values of 4 to maximal 25% were achieved for intensities and S/N ratios. This indicates that the reproducibility can be increased by performing pro-

filing experiments under standard temperature and atmospheric humidity conditions.

Conclusions

Any new technology, particularly one being presented as a potential clinically used diagnostic tool, requires stringent quality control to evaluate analytical performance over time. Instrument performance, however, must be compared not only during one experiment, but also over the course of time. We recently defined a standard protocol for calibration and acceptance criteria for the independent certified QC samples were established (32). Stringent QC as indicated above prevents unreliable data acquisition from the very start.

By introducing standard protocols and strict quality control, the analytical variation of protein profiling experiments can be significantly reduced. SELDI-TOF-MS still seems a promising technique for biomarker detection, and if reproducibility of SELDI-TOF-MS protein profiling can be further improved it can also become a valuable diagnostic tool in different diseases.

PRIMENA TEHNIKE *SELDI-TOF-MS* U PROFILISANJU PROTEINA: PREDNOSTI I ZAMKE

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Kratak sadržaj: Profilisanje proteina seruma tehnikom *Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF-MS)* čini se kao veoma važno dijagnostičko sredstvo za čitav niz bolesti. Senzitivnost i specifičnost koje se postižu ovom novom tehnologijom često su superiorne u odnosu na rezultate postignute uz pomoć drugih biomarkera. Međutim, njihova reproduktivnost i standardizacija još uvek su problematične. Objasnićemo tehniku *SELDI-TOF-MS* i razmotriti neke važne aspekte u izučavanju proteoma, kako pre tako i posle analize, kao i postupke za kontrolu kvaliteta. U ovom pregledu takođe su predstavljeni i naši podaci o otkrivanju biomarkera za postavljanje dijagnoze sarkoidoze.

Ključne reči: pre-analitički aspekti, post-analitički aspekti, proteomi, reproducibilnost, sarkoidoza, SELDI-TOF-MS, kontrola kvaliteta

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